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Filed : March 30, 2004

AMENDMENTS TO THE CLAIMS

1.-70. (Cancelled)

71. (Previously presented) A method of rapidly introducing an exogenous molecule into a live population of cells, comprising the steps of:

providing a live population of cells in the presence of said exogenous molecule;

providing an energy source configured to deliver a beam of radiation;

positioning a container holding said live population of cells within the focus of said beam of radiation; and

contacting one or more cells of said live population of cells with said beam of radiation, wherein said beam of radiation has a diameter of at least 2 μm at the point of contact with said one or more cells;

wherein one or more cells of said live population of cells is transiently permeabilized to allow said exogenous molecule to enter.

72. (Currently amended) The method of Claim 71, wherein said diameter of said beam of radiation is at least ~~5, 7, 10, 15, 20, 25, or 30~~ 10 μm at said point of contact with said live population of cells.

73. (Currently amended) The method of ~~Claims 71 or~~ Claim 72, wherein said beam of radiation delivers at most 2 $\mu\text{J}/\mu\text{m}^2$.

74. (Currently amended) The method of ~~Claims 71 or~~ Claim 72, wherein said beam of radiation delivers at most ~~1.5, 1, 0.7, 0.5, 0.3, 0.2, 0.1, 0.05, 0.02, 0.01 or 0.005~~ 0.2 $\mu\text{J}/\mu\text{m}^2$.

75. (Currently amended) The method of any of Claims 71-74, wherein said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5.

76. (Currently amended) The method of any of Claims 71-74~~Claim 75~~, wherein said lens has a numerical aperture of at most ~~0.4, 0.3, or~~ 0.25.

77. (Cancelled)

78. (Previously presented) The method of Claim 75, wherein said lens has an F-theta flat-field correction.

79. (Previously presented) The method of Claim 76, wherein said lens has an F-theta flat-field correction.

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80. (Currently amended) The method of Claim 75, wherein said lens comprises a working distance of at least ~~5, 7, or~~ 10 mm.

81. (Currently amended) The method of Claim 76, wherein said lens comprises a working distance of at least ~~5, 7, or~~ 10 mm.

82. (Currently amended) The method of Claims 71 or 72, wherein the viability of the transiently permeabilized cells is more than ~~50%, 60%, 70%, 80%, 90%, 95%, or 98%~~ after the method is performed.

83-84 (Cancelled)

85. (Previously presented) The method of Claim 71, wherein said live population of cells comprises a eukaryotic cell.

86-87. (Cancelled)

88. (Currently amended) The method of Claims 71 or 72, wherein contacting one or more cells of said live population of cells with said beam of radiation comprises contacting at least ~~5,000, 10,000, 20,000, 50,000, 70,000, 100,000, or 150,000~~ cells per minute with said beam of radiation.

89. (Currently amended) The method of any of Claims 71-~~74~~, wherein contacting one or more cells of said live population of cells with said beam of radiation further comprises the steps of:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said container holding said live population of cells.

90. (Previously presented) The method of Claim 89, wherein said pulsed beam of radiation is wider than any individual cell and is directed to the general area of said live population of cells.

91. (Previously presented) A method of rapidly introducing exogenous molecules into a live population of cells, comprising the steps of:

providing a live population of cells in the presence of exogenous molecules;

providing an energy source configured to deliver a beam of radiation;

positioning a container holding said live population of cells within the focus of said beam of radiation; and

contacting one or more cells of said live population of cells with said beam of radiation, wherein said beam of radiation delivers at most $2 \mu\text{J}/\mu\text{m}^2$;

wherein one or more cells of said live population of cells is transiently permeabilized to allow said exogenous molecules to enter.

92. (Currently amended) The method of Claim 91, wherein said beam of radiation delivers a value selected from the group of at most ~~1.5, 1, 0.7, 0.5, 0.3, 0.2, 0.1, 0.05, 0.02, 0.01 or 0.005~~ 0.2 $\mu\text{J}/\mu\text{m}^2$.

93. (Currently amended) The method of any of Claims 91-92, wherein said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5.

94. (Currently amended) The method of any of Claims 91-92 ~~Claim 91~~, wherein said lens has a numerical aperture selected from the group of at most ~~0.4, 0.3, or~~ 0.25.

95. (Currently amended) The method of ~~Claims 93 or 94~~ Claim 93, wherein said lens has an F-theta flat-field correction.

96. (Currently amended) The method of ~~Claims~~ Claim 91, wherein contacting said one or more cells of said live population of cells with said beam of radiation comprises:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said container holding said live population of cells.

97. (Previously presented) The method of Claim 96, wherein said pulsed beam of radiation is wider than any individual cell and is directed to the general area of said live population of cells.

98. (Cancelled)

99. (Currently amended) The method of Claims 91 or 92, wherein the viability of the transiently permeabilized cells is more than ~~50%, 60%, 70%, 80%, 90%, 95%, or 98%~~ after the method is performed.

100. (Currently amended) The method of ~~Claims 93 or 94~~ Claim 93, wherein the lens comprises a working distance of at least ~~5, 7, or 10~~ mm.

101. (Currently amended) The method of Claims 91 or 92, wherein contacting one or more cells of said live population of cells with said beam of radiation comprises contacting at

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least 5,000, ~~10,000, 20,000, 50,000, 70,000, 100,000, or 150,000~~ cells per minute with said beam of radiation.

102. (Currently amended) The method of ~~Claims 91 or 92~~ Claim 91, wherein said exogenous molecules comprises a nucleic acid, a polypeptide, a carbohydrate, a lipid or a small molecule.

103. (Previously presented) A method of rapidly introducing exogenous molecules into a live population of cells, comprising the steps of:

providing a live population of cells in the presence of exogenous molecules;

providing an energy source configured to deliver a beam of radiation;

positioning a container holding said live population of cells within the focus of said beam of radiation; and

contacting one or more cells of said live population of cells with said beam of radiation, wherein said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5;

wherein one or more cells of said live population of cells is transiently permeabilized to allow said exogenous molecules to enter.

104. (Currently amended) The method of Claim 103, wherein said lens has a numerical aperture selected from the group of at most ~~0.4, 0.3, or~~ 0.25.

105. (Cancelled)

106. (Currently amended) The method of Claims 103 or 104, wherein contacting said live population of cells with said beam of radiation comprises:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said live population of cells.

107. (Previously presented) The method of Claim 106, wherein said pulsed beam of radiation is wider than any individual cell and is directed to the general area of said live population of cells.

108. (Currently amended) The method of Claims 103 or 104, wherein contacting one or more cells of said live population of cells with said beam of radiation comprises contacting at

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least ~~5,000, 10,000, 20,000, 50,000, 70,000, 100,000, or 150,000~~ cells per minute with said beam of radiation.

109. (Previously presented) The method of Claims 103 or 104, wherein said lens has an F-theta flat-field correction.

110. (Currently amended) The method of Claims 103 or 104, wherein said lens comprises a working distance of at least ~~5, 7, or~~ 10 mm.

111. (Currently amended) The method of Claims 103 or 104, wherein the viability of the transiently permeabilized cells is more than ~~50%, 60%, 70%, 80%, 90%, 95%, or 98%~~ after the method is performed.

112-113 (Cancelled)

114. (Currently amended) A method of rapidly introducing an exogenous molecule into a live cell, comprising:

providing a live cell in the presence of said exogenous molecule;

providing an energy source configured to deliver a beam of radiation; and

contacting said live cell with said beam of radiation, wherein said live cell is transiently permeabilized to allow said exogenous molecule to enter; and

wherein (a) said beam of radiation has a diameter of at least ~~2- μ m~~ 10 μ m at the point of contact with said live cell, ~~(b) said beam of radiation delivers at most 2 μ J/ μ m²;~~
~~(c) said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5, or~~ (d) two or more of (a), (b) or (c).

115. (Currently amended) The method of Claim 114, wherein said beam of radiation has a diameter of at least ~~2- μ m~~ 15 μ m at the point of contact with said live cell.

116. (Currently amended) The method of Claim 115, wherein said diameter of said beam of radiation is at least ~~5, 7, 10, 15, 20, 25, or 30~~ μ m at the point of contact with said live cell.

117. (Previously presented) The method of Claim 114, wherein said beam of radiation delivers at most 2 μ J/ μ m².

118. (Currently amended) The method of Claim 117, wherein said beam of radiation delivers at most ~~1.5, 1, 0.7, 0.5, 0.3, 0.2, 0.1, 0.05, 0.02, 0.01 or 0.005~~ μ J/ μ m².

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119. (Previously presented) The method of Claim 114, wherein said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5.

120. (Currently amended) The method of Claim 119, wherein said lens has a numerical aperture of at most ~~0.4, 0.3, or~~ 0.25.

121. (Previously presented) The method of Claim 114, wherein said beam of radiation is wider than the cell.

122. (Previously presented) The method of Claim 114, wherein said beam of radiation is directed to the general area of said cell.

123. (New) The method of Claim 114, wherein said beam of radiation delivers at most $2 \mu\text{J}/\mu\text{m}^2$ and said beam of radiation is delivered through a lens with a numerical aperture of at most 0.5.

124. (New) The method of Claim 114, wherein said beam of radiation has a diameter of at least $25 \mu\text{m}$ at the point of contact with said live cell.

125. (New) The method of any one of Claims 116 or 124, wherein said beam of radiation delivers between $0.01 \mu\text{J}/\mu\text{m}^2$ and $0.1 \mu\text{J}/\mu\text{m}^2$.

126. (New) The method of any one of Claims 116 or 124, wherein said beam of radiation delivers at most $0.05 \mu\text{J}/\mu\text{m}^2$.

127. (New) The method of any one of Claims 116 or 124, wherein said beam of radiation delivers at most $0.1 \mu\text{J}/\mu\text{m}^2$.

128. (New) The method of any one of Claims 116 or 124, wherein said lens has a numerical aperture of at most 0.25.

129. (New) The method of Claim 125, wherein said lens has a numerical aperture of at most 0.25.

130. (New) The method of Claim 126, wherein said lens has a numerical aperture of at most 0.25.

131. (New) The method of Claim 127, wherein said lens has a numerical aperture of at most 0.25.

132. (New) The method of Claim 125, wherein contacting one or more cells of said live population of cells with said beam of radiation comprises contacting at least 5,000 cells per minute with said beam of radiation.

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133. (New) The method of Claim 125, wherein contacting one or more cells of said live population of cells with said beam of radiation comprises contacting at least 50,000 cells per minute with said beam of radiation.

134. (New) The method of Claim 71, wherein said diameter of said beam of radiation is at least 20 μm at said point of contact with said live population of cells.

135. (New) The method of Claim 94, wherein said lens has an F-theta flat-field correction.

136. (New) The method of Claim 92, wherein contacting said one or more cells of said live population of cells with said beam of radiation comprises:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said container holding said live population of cells.

137. (New) The method of Claim 93, wherein contacting said one or more cells of said live population of cells with said beam of radiation comprises:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said container holding said live population of cells.

138. (New) The method of Claim 94, wherein contacting said one or more cells of said live population of cells with said beam of radiation comprises:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said container holding said live population of cells.

139. (New) The method of Claim 95, wherein contacting said one or more cells of said live population of cells with said beam of radiation comprises:

pulsing said energy source; and

steering the pulsed beam of radiation from said energy source to multiple spots within said container holding said live population of cells.

140. (New) The method of Claim 94, wherein the lens comprises a working distance of at least 7 mm.